HUNTINGDON LIFE SCIENCES LTD
WOOLLEY RD, ALCONBURY
HUNTINGDON
CAMBRIDGESHIRE
PE17 5HS
ENGLAND

Report No.:

Title: Acute Inhalation Study of
in the Rat *via* Whole-Body Exposure.

Study No.:

External Testing Facility No.:

Test Substance:

Study Director:

Sponsor:

Sponsor Representative:

Testing Facility: Huntingdon Life Sciences Ltd,
Woolley Road,
Huntingdon,
Cambridgeshire,
PE17 5HS,
ENGLAND.

Study Completion Date: 22 June 2000

Security Statement:

TABLE OF CONTENTS

Page

V. FIGURE

VI. TABLES

VII. APPENDICES

ABSTRACT

The study was designed to assess the potential inhalation toxicity of when administered to the rat *via* whole-body exposure.

The procedure used is described in this report. The procedure complies with that described in the EPA Health Effects Testing Guidelines, Subpart B-General Toxicity Testing § 798.1150 Acute inhalation, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA). The study met the requirements of OPPTS 870.1300.

The albino rat (Sprague-Dawley) was chosen as the species as it has been shown to be a suitable model for this type of study and is the species recommended in the test guidelines.

Five groups of 5 male and 5 female rats were exposed to an aerosol produced from
A further group of 5 male and 5 female rats, acting as controls, was exposed to air only.

The mean exposure concentrations of the aerosol produced from were 0.515, 1.06, 1.49, 2.44 and 5.75 mg/l of air.

The LC_{50} for the test aerosol was between 1.49 and 2.44 mg/l.

GLP COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards as set forth in:

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No. 654) and, from 14 December 1999, the UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No. 3106).

EC Council Directive, 87/18/EEC of 18 December 1986, (Official Journal No. L 15/29) and from 1 May 1999.EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

The raw data has been reviewed by the Study Director, who certifies that the information contained in this report is consistent with and supported by the raw data.

22 June 2000

Date

Study Director,
Huntingdon Life Sciences Ltd.

QUALITY ASSURANCE STATEMENT

Study Title: Acute Inhalation Study of in the Rat *via* Whole-Body Exposure

Huntingdon Life Sciences
Study Number:

Study Director:

This study has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management, Huntingdon Life Sciences are given below:

Study Phase	Date of Inspection	Findings reported to: Study Director & Management
Protocol review	6-7 October 1998	7 October 1998
Bodyweight Accommodation & Husbandry Test Substance Control Exposure Procedures Aerosol Analysis Record Audits	5 & 9 November 1998	10 November 1998

Study Phase	Date of Inspection	Findings reported to: Study Director & Management
Post Mortem) Records Audit)	23 November 1998	24 November 1998
Exposure Procedures) Test Substance Control) Aerosol Analysis) Clinical Signs) Formulation Records) Records Audit)	10 March 1999	11 March 1999
Post Mortem)	24 March 1999	24 March 1999
Post Mortem) Records Audit)	6 & 7 April 1999	8 April 1999
Report/data audit	20 March 2000	21 March 2000

16 June 2000

Date

Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

APPROVAL SIGNATURES

This report consist of Pages 1 through 51 including Tables 1-9 and Appendices 1 - 3.

22 June 2000

Date

Management,
Huntingdon Life Sciences Ltd.

22 June 2000

Date

Study Director,
Huntingdon Life Sciences Ltd.

29 June 2000

Date

Sponsor Representative,

STUDY INFORMATION

Study Initiation Date:	5 October 1998
Experimental Start Date:	9 November 1998
Experimental Termination Date:	6 April 1999
Study Completion Date:	22 June 2000
Study Director:	
Sponsor Representative:	
Sponsor:	
Senior Technician for study:	
Toxicologist:	
Chief Technician – Inhalation Toxicology:	
Director of Quality Assurance:	
Head of Veterinary Services:	

I. INTRODUCTION

The study was designed to assess the potential inhalation toxicity of when administered to the rat *via* whole-body exposure

The procedure used is described in this report. The procedure complies with that described in the EPA Health Effects Testing Guidelines, Subpart B-General Toxicity Testing § 798.1150 Acute inhalation, September 27, 1985 (described in Federal Register Vol. 50, No. 188) and subsequent revisions. Subpart B provides detailed information relating to data requirements of 40 CFR Part 798 and supports the Toxic Substances Control Act (TSCA). The study met the requirements of OPPTS 870.1300

The protocol was approved by the Study Director and Huntingdon Life Sciences Management on 5 October 1998 and by the Sponsor's Representative on 25 September 1998.

The albino rat (Sprague-Dawley) was chosen as the species as it has been shown to be a suitable model for this type of study and is the species recommended in the test guidelines.

The rats were dosed by exposure to a respirable aerosol as this is the route of exposure required by the test guideline and method. Exposure by inhalation is an anticipated route of human exposure.

II. MATERIALS AND METHODS

A. Test Substance: lot number BN028339, was received at Huntingdon Life Sciences on 6 April 1998. The test substance a pale yellow liquid, was stored at room temperature and was regarded as the 'pure' material representative of The Huntingdon Test Substance Data Sheet indicated that the test substance was stable until 28 February 2001. A 1 g archive sample was retained by Huntingdon Life Sciences.

B. Animals: Ten male and 10 nulliparous and non-pregnant female albino rats (Sprague-Dawley in origin (Crl: (IGS)CD BR), approximately 7 and 8 weeks old respectively, were selected from a consignment of rats obtained from Charles River UK Limited, Manston Road, Margate, Kent, England on the 28 October 1998. Further groups of rats were selected from consignments arriving from the same source on 3 and 10 March 1999. On arrival, each animal was allocated to a group, each of 5 males and 5 females. The animals were identified individually by a number tattooed on the ear pinnae as follows:

Group	Identity numbers	
	Male	Female
1	61-65	66-70
2	71-75	76-80
3	81-85	86-90
4	91-95	96-100
5	21-25	26-30
6	1-5	6-10

The rats were housed by sex in groups of 5 and acclimatised to laboratory conditions for at least 7 days before the day of the exposure.

C. Food and Water: Rat and mouse diet 1 (RM1, SDS Limited, Witham, Essex, England) was provided *ad libitum* from hoppers attached to the holding cages, and drinking water (Anglian Water Services Limited) was provided *ad libitum* from polypropylene bottles. Food and water were not available during the exposure period.

The batches of diet used for the study were analysed once by the supplier for nutrients, possible contaminants or micro-organisms likely to be present in the diet, and which, if in excess may have had an undesirable effect on the test system. Clearance for use certificates are appended to this report.

Results of routine physical and chemical analysis of drinking water performed by the supplier were made available to Huntingdon Life Sciences Ltd. as quarterly summaries. A representative of the results is appended to this report. Water was supplied in conformity with EC directive 80/7788/EEC and UK Water Act 1989 and subsequent amendments. A representative certificate of analysis is appended to this report.

Samples of water were taken from the drinking water source in the animal rooms at approximately six monthly intervals. These samples were analysed for microbial contaminants (total viable count, coliform count and *E.Coli* count) by Huntingdon Life Sciences Department of Cellular Toxicology. A certificate of analysis is appended to this report.

D. Housing and Environment: The holding cages (size 35cm × 53 cm × 25 cm height) were made of stainless steel sheet and wire mesh and were suspended on a movable rack. Each cage was identified by a coloured label displaying the study number, treatment group descriptor, sex and identity numbers of the animals therein. While in their cages all rats had free access to an excess amount of SDS rat and mouse diet (RM1) and tap water.

The rats remained in a holding room except for the 4-hour exposure and an overnight post exposure period when the rats in the test groups were kept in a ventilated cabinet to allow dispersal of any residual test substance.

The temperature and relative humidity (RH) of the holding area were recorded using a Kent Clearspan recorder. Air extraction was *via* a balanced system providing 12 - 15 air changes per hour. The study holding room conditions were generally maintained within the environmental control settings of 21°C ± 2°C and 55% ± 10%. Any excursions outside of the set ranges were small and of short duration. Details are archived with the study data. None of the excursions were considered to have had any impact on the outcome of the study.

Room lighting was by artificial light between 07.30 and 19.30 daily and controlled automatically.

E. Methods:

1. Animals: The animals were manually selected and randomly allocated to groups. The weight variation within groups did not exceed ±20% of the mean weight for each sex.

2. Animal welfare: The in-life experimental procedures to be undertaken during the course of this study were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 (the Act). The Act, administered by the UK Home Office, regulates all scientific procedures in living animals which may cause pain, suffering, distress or lasting harm and provides for the designation of establishments where procedures may be undertaken, the licensing of trained individuals who perform the practical techniques and the issue of project licences for specified programmes of work. This study complied with all applicable sections of the Act and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures and the Humane Killing of Animals under Schedule 1 to the Act, issued under section 21 of the Act.

3. Inhalation exposure

Groups of rats (5 males and 5 females) were exposed continuously for 4 hours to an aerosol generated from The target concentrations were:

Group	Date of exposure	Target concentration (mg/l)
1	9 November 1998	Air control
2	9 November 1998	5
3	10 March 1999	2
4	17 March 1999	0.5
5	18 March 1999	1
6	23 March 1999	1.5

The mean chamber concentration of the aerosol for each of the test groups is presented in the **RESULTS** section of this report.

Generation of the test atmosphere – The test substance was metered at a constant rate from a polypropylene syringe mounted on a syringe pump (Precidor® type 5003) to a stainless steel concentric jet atomiser. The aerosol produced passed through a 2 piece glass elutriator prior to entering the exposure chamber. A diagram of the system is presented in Figure 1.

During the first exposure with animals the test substance was observed to form a solid substance on contact with water. It was considered that the water vapour produced by the animals during exposure resulted in most of the in the chamber being converted to an aerosol of this solid.

The air supplied for the generation system was obtained from a compressed air line, and was filtered, dried and oil-free.

Exposure chambers - The whole-body exposure chambers were of square section (51 cm x 51 cm x 38 cm high) and were constructed of acrylic polymer. The chambers were fitted with a pyramidal top section with an enclosed volume of approximately 120 litres. The rats were contained for exposure in a stainless steel mesh exposure cage subdivided to provide 10 individual compartments.

The chambers were operated under dynamic airflow conditions. The flow rate of the test atmosphere to the exposure chamber was 25 l/min. The chamber atmosphere flow rate was monitored continuously and recorded at 30 minute intervals during exposure.

The test atmosphere entered at the top centre of the pyramidal top of the exposure chamber and was extracted through a perforated base, below the level of the rats. The exposure chamber was contained within a large extract cabinet exhausting to atmosphere through an absolute filter.

The temperature and relative humidity were monitored continuously during exposure and recorded at 30 minute intervals. Temperature was measured with an alcohol bulb thermometer and relative humidity with an ADC Water Vapour Analyser.

Atmosphere analysis - At least 5 samples of air were removed from the test chamber at intervals during each exposure in order to determine the concentration of the test aerosol. Additional samples were obtained as necessary to monitor the chamber concentration following adjustments to the exposure system.

Following the first exposure each air sample was withdrawn, at a rate of 2 litres per minute, through a pre-weighed glass fibre filter (Whatman GF/A) mounted in an open face filter holder. The filters were re-weighed following sampling for gravimetric analysis of the test aerosol. The volume of air sampled was measured using a wet-type gas meter (Model DM3B, G H Zeal Ltd., London, England).

For the first test group exposure only, in addition to sample collected on glass fibre filters, attempts were made to collect samples using sintered glass bubblers containing toluene as trapping agent to determine the actual concentration by chemical analysis. A method of analysis had been developed during preliminary generation trials without animals. However, attempts were unsuccessful and it was determined that the aerosol of formed a solid substance on contact with water vapour in air, which could not be analysed chemically with the method established. Therefore, analysis of the test atmosphere produced from the test substance was analysed by gravimetric means only. No further reference to chemical analysis will be made in this report. Details of the method and method development are archived with the study data.

Particle size determination - Two additional air samples were taken during the exposure, at a sampling rate of 2 litres per minute, using a Marple cascade impactor (Model 296, Graseby Andersen Inc., Atlanta, GA, USA). The volume of air sampled was measured using a wet-type gas meter.

The amount of test material collected on the stages of the sampler was determined gravimetrically. The particle size distribution of the test atmosphere was assessed using linear regression analysis of the probit of the cumulative percentage of the total particles collected, smaller than the cut-point of each stage, against the logarithm of the cut-point of each stage.

The collection characteristics for the Marple sampler are shown in Table 2.

Nominal concentration - The nominal concentration of the test substance was calculated from the amount of test substance delivered to the atomiser and the total volume of air flowing through the exposure system during the period of generation.

7. Clinical signs: The rats were observed continuously for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period.

   The clinical signs were recorded at the end of the chamber equilibration period, 0.25, 0.5 and 1.0 hours into exposure then at hourly intervals during the remainder of the exposure. Signs were also recorded immediately after exposure and at 1 and 2 hours after exposure.

   During the observation period, the clinical signs were recorded once in the morning and then as necessary following a later check for survival.

8. Bodyweight: All rats were weighed at least twice during the week prior to exposure, at Time 0 (immediately before exposure) and weekly during the observation period.

9. Food consumption: The amount of food consumed by each cage of rats was measured from weighday to weighday throughout the study.

10. Water consumption: The amount of water consumed by each cage of rats was measured daily from Day 2 of the observation period following the gross observation of reduced consumption in test group rats.

11. Termination: Rats surviving the observation period were killed by intraperitoneal injection of pentobarbitone sodium and exsanguinated when clinically dead. A complete macroscopic examination of each rat was performed. The lungs (including the larynx and trachea), liver and kidneys were dissected free, weighed and the weights recorded. The kidneys were weighed together. The tissues were then discarded. Four uterine masses discovered in Animal 90F were preserved.

F. Location of Study records: The protocol and amendments, raw data, specimens, a sample of the test substance and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd., together with a copy of the final report are lodged in the Huntingdon Life Sciences Ltd., Archive, Huntingdon, England. Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's prior approval.

G. Statistical analysis: None

H: Deviations from the study protocol: Minor deviations from the study protocol had no impact on the outcome of the study.

The deviations comprised:

- lack of Study Director and Home Office Licencee initials on the cage labels.
- in order to comply with recommendations of UK Home Office Code of Practice for the Housing and Care of Animals the rats were housed 5 to a cage.
- with so few animals the use of a computer programme to allocate the animals was unnecessary.
- bodyweights for Group 6 animals were recorded 8 rather than within 7 days prior to exposure.
- animals health status was reviewed by an experienced Senior Animal Technician.
- on 28 March 1999 the animal room timeclocks were set to British Summer Time resulting in an 11 hour light period on that day.

III. RESULTS

A. Chamber atmosphere conditions

Chamber concentration of test aerosol – The results for the air samples collected during the exposures are presented in Table 1.

The data are summarised as follows:

Group	Target concentration (mg/l)	Analysed concentration (mg/l)	Nominal concentration (mg/l)
1	Air control	0	-
2	5	5.75	19.79
3	2	2.44	9.71
4	0.5	0.515	1.55
5	1	1.06	3.59
6	1.5	1.49	6.36

The degree to which water vapour produced by the breathing of the animals would convert aerosol to the solid product was somewhat unpredictable and therefore the early exposure concentrations exceeded the target.

Differences between the analysed and nominal concentrations reflect an expected degree of loss of the test material due to impaction and deposition within an exposure system of the type used.

Particle size distribution

The data are presented in Table 2 and are summarised as follows:

Group	MMAD (σg)	% < 7 μm ad
2	1.2 (2.56)	96
3	1.3 (2.25)	98
4	0.9 (2.25)	99
5	0.9 (2.22)	99
6	1.0 (2.20)	99

MMAD mass median aerodynamic diameter
σg geometric standard deviation
ad aerodynamic diameter

Particles less than 7 μm aerodynamic diameter are considered to be respirable to the rat. The test aerosols were completely respirable.

Chamber air temperature and relative humidity

The data are summarised as follows:

Group	Temperature (°C)		Relative humidity (%)	
	Mean	Sd	Mean	sd
1	21.9	0.17	54	3.0
2	22.4	0.85	54	4.4
3	21.8	0.83	39	1.7
4	21.9	1.45	54	3.1
5	21.7	0.56	52	8.1
6	22.4	0.60	61	6.3

The differences between groups were small were considered to have had no effect on the outcome of the study.

B. Mortality

The data are summarised as follows:

Group	Analysed concentration (mg/l)	Mortality		
		Male	Female	Total
1	0	0/5	0/5	0/10
2	5.75	5/5	4/5	9/10
3	2.44	5/5	3/5	8/10
4	0.515	0/5	0/5	0/10
5	1.06	0/5	0/5	0/10
6	1.49	0/5	0/5	0/10

For Group 2 all 5 males and 2 females were found dead on Day 1 of the observation period, 1 female was found dead on Day 2 and 1 on Day 3.

For Group 3 all 5 males and 2 females were found dead on Day 1, 1 female was found dead on Day 2.

All Group 1 animals survived.

C. Clinical Signs:

During exposure – The incidence of signs observed during exposure is presented in Table 3.

Signs related to exposure to the test aerosol comprised exaggerated breathing and partially closed eyes in all test groups and reduced motor activity in Groups 3 to 6.

During the observation period - The incidence of signs observed during the observation period is presented in Table 4.

Irregular, noisy and/or exaggerated breathing was seen in all test groups. Partially closed eyes were seen in Groups 2, 3, 5 and 6. Lethargy was seen in Groups 2 and 3. Ataxia was observed in the 2 female rats from Group 3 surviving until the end of the observation period.

D. Body Weights: The data are presented in Table 5.

A dose related reduction in body weight gain over the observation period was seen in animals from Groups 4, 5 and 6 in comparison with controls.

E. Food consumption: The data are presented in Table 6.

Consumption by test groups was lower than that of the control group during the observation period.

F. Water consumption: The data are presented in Table 7.

Consumption by groups 4, 5 and 6 was generally comparable to that of the control group during the observation period

G. Macroscopic pathology: The data are presented in Table 8:

Severely congested lungs were seen in all decedent animals. The observation is considered to be associated with the cause of death. Areas of severe congestion together with pale raised hardened areas were also seen in the surviving female exposed to 5.75 mg/l.

Pale raised areas were also seen in the lungs of a proportion of surviving rats of both sexes in all other groups exposed to the test aerosol.

H. Organ weights: The data are presented in Table 9.

Surviving female rats exposed to 5.75 or 2.44 mg/l had lung weights greater than control rats. In all other surviving animals from other test groups there were no differences in organ weights that were considered to be a direct effect of exposure to the test aerosol.

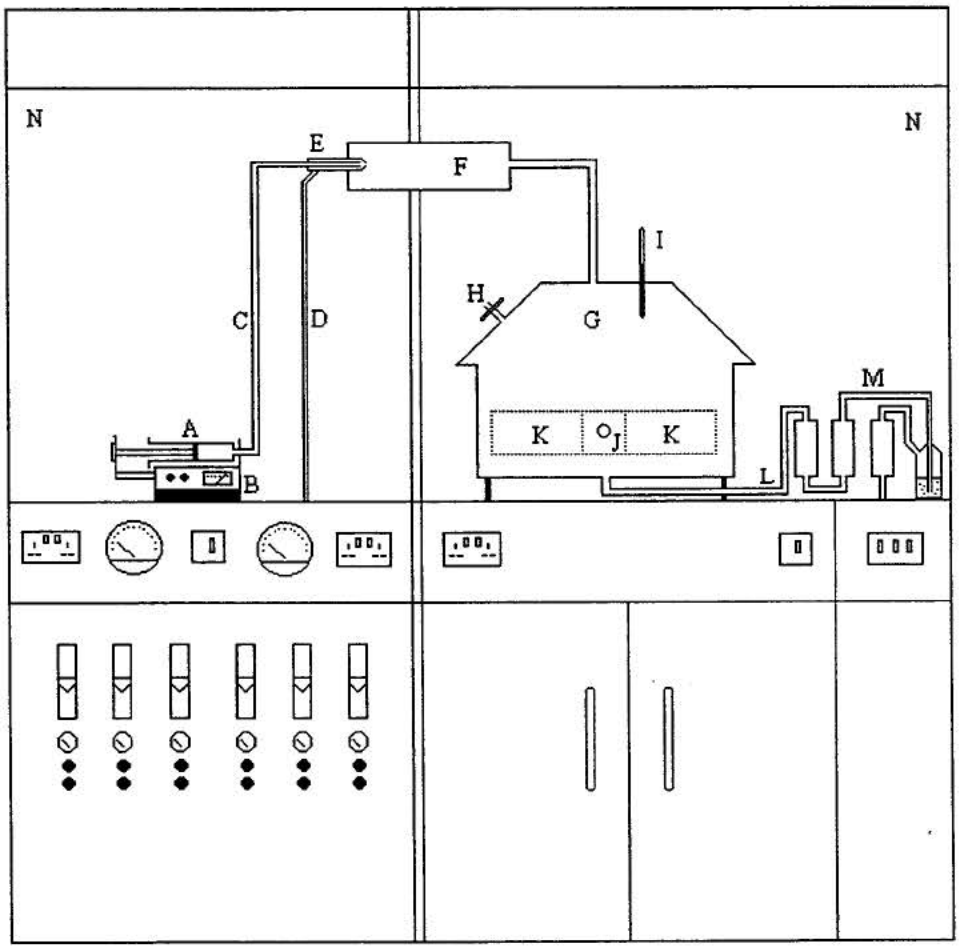
Lower liver weights seen in test group survivors were considered to be a secondary effect of reduced bodyweight gain.

IV. CONCLUSION

The inhalation toxicity curve for this material is very steep and the LC_{50} for the aerosol produced from was between 1.49 and 2.44 mg/l. Further exposures were considered to be unnecessary. The difference in concentrations between that causing zero and 80% mortality was such that it was considered that the fine degree of control of chamber concentration necessary to refine the prediction of the LC_{50} could not be achieved given the nature of the test material.

FIGURE 1

Exposure system



A Test substance (in syringe)
B Syringe pump
C PTFE feed line
D Atomiser air supply
E Jet concentric atomiser
F Elutriator
G Exposure chamber (120 litres)
H Sample line to water vapour analyser
I Thermometer
J Sampling port
K Rat holding cage
L Extract from exposure chamber
M Filter/extract unit
N Air extraction cabinet

TABLE 1

Chamber concentration of test aerosol

Sample	Concentration of test aerosol (mg/l)				
	Group				
	2	3	4	5	6
1	B	F[1]	0.511	0.950	1.32
2	B	2.78	0.519	1.19	1.39
3	B	1.46	0.521	1.21	1.57
4	B	2.61	0.510	1.03	1.59
5	B	2.55	0.515	0.995	1.56
6	5.12	2.72		0.982	1.53
7	B	F[2]			
8	6.11	2.54			
9	6.01				
mean	5.75	2.44	0.515	1.06	1.49
sd	0.545	0.491	0.0048	0.112	0.111

B bubbler sample, unable to analyse solid material
F[1] sample failure, filter not dried
F[2] sample failure, filter torn
sd Standard deviation

TABLE 2

Particle size distribution of test aerosol

Gravimetric Analysis

Group	Sample	Stage	Cut-off size (μm)	Amount collected (mg)
2 (5.75)	PSD 1	3	9.8	0.04
		4	6.0	0.21
		5	3.5	0.26
		6	1.55	0.97
		7	0.93	0.39
		8	0.5	0.06
		Filter	0.0	0.15
			Totals	2.08
	PSD 2	3	9.8	0.05
		4	6.0	0.19
		5	3.5	0.19
		6	1.6	0.90
		7	0.9	0.35
		8	0.5	0.06
		Filter	0.0	0.21
			Totals	1.95

Calculations

Cut-off size (*μm*)	% less than size (cumulative)
9.8	98.8
6.0	93.8
3.5	87.8
1.55	58.7
0.93	42.7
0.5	15.7
MMAD	1.2 (μm)
σg	2.56
% respirable (<7 μm)	96

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of test aerosol - continued)

Gravimetric Analysis

Group	Sample	Stage	Cut-off size (µm)	Amount collected (mg)
3 (2.44)	PSD 1	3	9.8	0.01
		4	6.0	0.20
		5	3.5	0.42
		6	1.55	1.24
		7	0.93	0.39
		8	0.5	0.07
		Filter	0.0	0.36
			Totals	2.69
	PSD 2	3	9.8	0.01
		4	6.0	0.31
		5	3.5	0.52
		6	1.55	1.41
		7	0.93	0.50
		8	0.5	0.14
		Filter	0.0	0.40
			Totals	3.29

Calculations

Cut-off size (*µm*)	% less than size (cumulative)
9.8	99.8
6.0	94.2
3.5	84.7
1.55	55.7
0.93	39.1
0.5	14.2
MMAD	1.3 (µm)
σg	2.25
% respirable (<7 µm)	98

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of test aerosol - continued)

Gravimetric Analysis

Group	Sample	Stage	Cut-off size (μm)	Amount collected (mg)
4 (0.515)	PSD 1	3	9.8	0
		4	6.0	0.05
		5	3.5	0.19
		6	1.55	0.97
		7	0.93	0.59
		8	0.5	0.12
		Filter	0.0	0.69
			Totals	2.61
	PSD 2	3	9.8	0
		4	6.0	0.05
		5	3.5	0.17
		6	1.6	0.91
		7	0.9	0.53
		8	0.5	0.22
		Filter	0.0	0.54
			Totals	2.42

Calculations

Cut-off size (*μm*)	% less than size (cumulative)
9.8	100.0
6.0	98.8
3.5	94.8
1.55	72.1
0.93	55.0
0.5	26.8
MMAD	0.9 (μm)
σg	2.25
% respirable (<7 μm)	99

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of test aerosol - continued)

Gravimetric Analysis

Group	Sample	Stage	Cut-off size (µm)	Amount collected (mg)
5 (1.06)	PSD 1	3	9.8	0.01
		4	6.0	0.05
		5	3.5	0.15
		6	1.55	0.97
		7	0.93	0.59
		8	0.5	0.13
		Filter	0.0	0.31
			Totals	2.21
	PSD 2	3	9.8	0
		4	6.0	0.05
		5	3.5	0.19
		6	1.6	0.91
		7	0.9	0.55
		8	0.5	0.05
		Filter	0.0	0.42
			Totals	2.17

Calculations

Cut-off size (*µm*)	% less than size (cumulative)
9.8	100.1
6.0	98.8
3.5	93.7
1.55	69.3
0.93	51.5
0.5	25.6
MMAD	0.9 (µm)
σg	2.22
% respirable (<7 µm)	99

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 2

(Particle size distribution of test aerosol - continued)

Gravimetric Analysis

Group	Sample	Stage	Cut-off size (µm)	Amount collected (mg)
6 (1.49)	PSD 1	3	9.8	0.02
		4	6.0	0.12
		5	3.5	0.39
		6	1.55	1.43
		7	0.93	0.77
		8	0.5	0.12
		Filter	0.0	0.33
			Totals	3.18
	PSD 2	3	9.8	0.01
		4	6.0	0.12
		5	3.5	0.33
		6	1.6	1.41
		7	0.9	0.78
		8	0.5	0.15
		Filter	0.0	0.45
			Totals	3.25

Calculations

Cut-off size (*µm*)	% less than size (cumulative)
9.8	99.9
6.0	97.9
3.5	92.0
1.55	66.4
0.93	46.8
0.5	20.4
MMAD	1.0 (µm)
σg	2.20
% respirable (<7 µm)	99

MMAD Mass median aerodynamic diameter
σg Standard geometric deviation

TABLE 3

Clinical signs during exposure

Group (mg/l)	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1M (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
2M (5.75)	Normal appearance and behaviour	5	5					
	Exaggerated breathing				5	5	5	5
	Eyes partially closed			5	5	5	5	5
3M (2.44)	Normal appearance and behaviour	5	5					
	Exaggerated breathing			5	5	5	5	5
	Eyes partially closed				5	5	5	5
	Reduced motor activity				5	5	5	5
4M (0.515)	Normal appearance and behaviour	5	5	3				
	Exaggerated breathing			2	5	5	5	5
	Eyes partially closed				2	5	5	5
	Reduced motor activity					5	5	5
5M (1.06)	Normal appearance and behaviour	5	5					
	Exaggerated breathing			5	5	5	5	5
	Eyes partially closed			5	5	5	5	5
	Reduced motor activity				5	5	5	5
6M (1.49)	Normal appearance and behaviour	5	5					
	Exaggerated breathing				5	5	5	5
	Eyes partially closed			5	5	5	5	5
	Reduced motor activity			5	5	5	5	5

* Clinical signs recorded during the equilibration period

TABLE 3

(Clinical signs during exposure - continued)

Group (mg/l)	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1F (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
2F (5.75)	Normal appearance and behaviour	5	5					
	Exaggerated breathing				5	5	5	5
	Eyes partially closed			5	5	5	5	5
3F (2.44)	Normal appearance and behaviour	5	5					
	Exaggerated breathing			5	5	5	5	5
	Eyes partially closed				5	5	5	5
	Reduced motor activity				5	5	5	5
4F (0.515)	Normal appearance and behaviour	5	5	5				
	Exaggerated breathing				5	5	5	5
	Eyes partially closed				4	5	5	5
	Reduced motor activity					5	5	5
5F (1.06)	Normal appearance and behaviour	5	5					
	Exaggerated breathing			5	5	5	5	5
	Eyes partially closed			5	5	5	5	5
	Reduced motor activity				5	5	5	5
6F (1.49)	Normal appearance and behaviour	5	5					
	Exaggerated breathing				5	5	5	5
	Eyes partially closed			5	5	5	5	5
	Reduced motor activity			5	5	5	5	5

* Clinical signs recorded during the equilibration period

TABLE 4

Clinical signs during observation period

Group (mg/l)	Signs	Number showing signs Day of observation period																
		*0hr	*1hr	*2hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2M (5.75)	Brown staining around snout/jaws	5	5	5														
	Eyes partially closed	5	5	5														
	Substance on fur (not visible but altered fur texture)	5	5	5														
	Lethargic	5	5	5														
	Irregular breathing	5	5	5														
	Brown staining on forepaws	5	5	5														
	Noisy respiration		3															
	Dead				5													
3M (2.44)	Brown staining around snout/jaws	5	5	5	3													
	Eyes partially closed	5	5	5														
	Substance on fur (tactile presence)	5	5	5	3													
	Lethargic	5	5	5	3													
	Exaggerated breathing	5	5	5	3													
	Brown staining on forepaws	1	4	4	3													
	Noisy respiration				4													
	Yellow staining UG area		5	5	4													
	Dead				5													

* Clinical signs recorded after exposure on day of exposure
UG Urogenital

TABLE 4

(Clinical signs during observation period - continued)

Group (mg/l)	Signs	Number showing signs Day of observation period															
		*0hr	*1hr	*2hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4M	Normal appearance and behaviour						3	5	5	5	5	5	5	5	5	5	5	5
(0.515)	Substance on fur (tactile presence)	5	5	5														
	Extremities cold to touch	5																
	Exaggerated breathing	5	5	5	5	5	2											
	Noisy respiration	1	3	3	3	2												
	Wet fur UG area	1	1	1														
5M	Normal appearance and behaviour									3	3	4	5	5	5	5	5	5
(1.06)	Substance on fur (tactile presence)	5	5	5	5													
	Brown staining around snout/jaws		3	4	3													
	Exaggerated breathing	5	5	5	5	5	5	5	5	2	2	1						
	Noisy respiration		1	1	3	2	2											
	Eyes partially closed	5																
	Yellow staining UG area	5	5	5	5	2	2	1	1									
	Brown staining forepaws			4	3													
	Brown staining whole body					4	4											
6M	Normal appearance and behaviour					1	1	4	5	5	5	5	5	5	5	5	5	5
(1.49)	Substance on fur (tactile presence)	5	5	5														
	Brown staining around snout/jaws	5	5	5	1													
	Exaggerated breathing	5	5	5	3	4	4											
	Noisy respiration					4	1											
	Eyes partially closed	5	5	5														
	Yellow staining UG area	5	5	5	4	3	2	1										

* Clinical signs recorded after exposure on day of exposure

UG Urogenital

TABLE 4

(Clinical signs during observation period - continued)

Group (mg/l)	Signs	Number showing signs Day of observation period																
		*0hr	*1hr	*2hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1F (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2F (5.75)	Brown staining around snout/jaws	5	5	5	3	2	1											
	Eyes partially closed	5	5	5														
	Substance on fur	5	5	5	3	2	1	1										
	Lethargic	5	5	5	3	2	1											
	Irregular breathing	5	5	5	3	2	1	1	1	1	1	1	1	1				
	Brown staining on forepaws	5	5	5	3	2	1											
	Noisy respiration			1	3	2	1	1	1	1	1	1	1	1				
	Whole body cold to touch				3	2	1											
	Hunched posture				1										1	1	1	1
	Yellow/brown staining UG area					2	1	1	1	1	1	1	1	1	1	1	1	1
	Sensitive to touch							1	1	1	1							
	Dead				2	1	1											
3F (2.44)	Normal appearance and behaviour							1	1	1	2	2	2	2	2	2	2	2
	Brown staining around snout/jaws	5	5	5	5	2	1											
	Eyes partially closed	5	5	5	1													
	Substance on fur (tactile presence)	5	5	5	5	2												
	Lethargic	5	5	5	5	2												
	Exaggerated breathing	5	5	5	5	2	2	1										
	Brown staining on forepaws	4	4	4	4	2												
	Noisy respiration				5	2	1	1	1	1								
	Yellow staining UG area				3	2	1	1	1									
	Ataxic			1		2	1	1										
	Pale extremities					2	2	1										
	Dead				2	1												

* Clinical signs recorded after exposure on day of exposure

UG Urogenital

TABLE 4

(Clinical signs during observation period - continued)

Group (mg/l)	Signs	Number showing signs Day of observation period															
		*0hr	*1hr	*2hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4F	Normal appearance and behaviour						5	5	5	5	5	5	5	5	5	5	5	5
(0.515)	Substance on fur (tactile presence)	5	5	5														
	Extremities cold to touch	5	5	5														
	Exaggerated breathing	5	5	5	5	5												
	Noisy respiration		1	2	2	1												
	Irregular breathing		2	2														
5F	Normal appearance and behaviour										4	5	5	5	5	5	5	5
(1.06)	Substance on fur	5	5	5	5	1	1											
	Extremities cold to touch			1														
	Brown staining around snout/jaws	2	4	5	5													
	Exaggerated breathing	5	5	5	5	5	5	5	5	5								
	Noisy respiration			1	2													
	Eyes partially closed	5																
	Yellow staining UG area	5	5	5	5	2	2	1	1	1	1							
	Brown staining forepaws			3	2													
	Brown staining whole body					2	2											
6F	Normal appearance and behaviour							5	5	5	5	5	5	5	5	5	5	5
(1.49)	Substance on fur (tactile presence)	5	5	5														
	Brown staining around snout/jaws	5	5	5														
	Exaggerated breathing	5	5	5	5	5	5											
	Noisy respiration				2	1												
	Eyes partially closed	5	5	5														
	Yellow staining UG area	5	5	5	4	4	1											
	Crusty around eyes				1													
	Brown staining on head				4	3												

* Clinical signs recorded after exposure on the day of exposure

UG Urogenital

TABLE 5

Individual and group mean body weights (g)

Group	Rat	Day of observation													Gain	Gain
		-11	-10	-8	-7	-6	-5	-4	-3	-2	-1	0	7	14	0-7	0-14
1M	61		234		265			292				330	377	421	47	91
(Control)	62		234		269			296				339	379	412	40	73
	63		221		252			280				313	367	405	54	92
	64		233		269			300				345	406	445	61	100
	65		218		254			281				322	387	438	65	116
	Mean		228		262			290				330	383	424	53	94
2M	71		223		253			282				312	-	-		
(5.75)	72		218		251			283				317	-	-		
	73		223		254			278				309	-	-		
	74		230		262			288				315	-	-		
	75		223		251			276				299	-	-		
	Mean		223		254			281				310	-	-		
3M	81						227			249	261	266	-	-		
(2.44)	82						224			254	264	270	-	-		
	83						207			234	240	250	-	-		
	84						216			244	253	259	-	-		
	85						216			240	247	254	-	-		
	Mean						218			244	253	260	-	-		
4M	91						230				272	276	307	353	31	77
(0.515)	92						227				265	275	309	361	34	86
	93						223				257	268	318	373	50	105
	94						221				260	263	293	337	30	74
	95						229				276	282	317	370	35	88
	Mean						226				266	273	309	359	36	86
5M	21					222			249			277	303	344	26	67
(1.06)	22					223			251			280	307	344	27	64
	23					220			250			277	309	369	32	92
	24					233			260			287	311	371	24	84
	25					216			245			274	291	349	17	75
	Mean					223			251			279	304	355	25	76
6M	1	226		249				282				314	316	360	2	46
(1.49)	2	231		255				289				323	329	382	6	59
	3	220		244				268				295	303	348	8	53
	4	226		257				295				326	335	379	9	53
	5	218		244				281				324	337	391	13	67
	Mean	224		250				283				316	324	372	8	56

TABLE 5

(Individual and group mean body weights (g) – continued)

Group	Rat	Day of observation													Gain	Gain
		-11	-10	-8	-7	-6	-5	-4	-3	-2	-1	0	7	14	0-7	0-14
1F	66		205		217			226				240	248	263	8	23
(Control)	67		193		206			208				224	245	265	21	41
	68		204		222			229				245	255	268	10	23
	69		200		213			222				230	254	258	24	28
	70		200		211			209				218	242	267	24	49
	Mean		200		214			219				231	249	264	18	33
2F	76		196		204			220				234	-	-		
(5.75)	77		208		224			239				249	-	-		
	78		200		210			216				228	-	-		
	79		192		197			215				228	166	164	-62	-64
	80		183		201			209				220	-	-		
	Mean		196		207			220				232	166	164		
3F	86						208			205	207	208	-	-		
(2.44)	87						215			225	220	226	-	-		
	88						205			215	219	216	205	239	-11	23
	89						190			206	206	209	-	-		
	90						210			206	210	211	222	254	11	43
	Mean						206			211	212	214	214	247	0	33
4F	96						185				188	186	194	202	8	16
(0.515)	97						202				216	220	232	247	12	27
	98						211				220	211	223	239	12	28
	99						214				226	219	233	244	14	25
	100						195				208	202	216	227	14	25
	Mean						201				212	208	220	232	12	24
5F	26					203			210			214	229	251	15	37
(1.06)	27					199			209			212	214	231	2	19
	28					193			203			211	205	223	-6	12
	29					197			206			212	212	227	0	15
	30					200			222			221	217	233	-4	12
	Mean					198			210			214	215	233	1	19
6F	6	200		210				208				211	215	218	4	7
(1.49)	7	196		199				201				210	217	224	7	14
	8	205		207				219				230	232	246	2	16
	9	203		214				221				228	225	237	-3	9
	10	202		214				215				226	211	216	-15	-10
	Mean	201		209				213				221	220	228	-1	7

TABLE 6

Food consumption

Period of consumption (Day)	Food consumption (g/rat/day)											
	1M (Control)	2M (5.75)	3M (2.44)	4M (0.515)	5M (1.06)	6M (1.49)	1F (Control)	2F (5.75)	3F (2.44)	4F (0.515)	5F (1.06)	6F (1.49)
-11 to -9						29						20
-10 to -8	34	32					23	22				
-8 to -5						30						21
-7 to -5	35	32					23	22				
-6 to -4					29						21	
-5 to -3			28						20			
-5 to -2				30						20		
-4 to -1	35	32				31	23	22				21
-3 to -1					30						20	
-2			29						19			
-1			30	31					20	18		
1 to 7	36	-	-	25	22	20	24	2	11	17	17	16
8 to 14	36	-	-	34	33	34	25	10	25	20	24	21
Cumulative (g/rat)												
-11 to -1						330						226
-10 to -1	347	322					228	219				
-6 to -1					177						125	
-5 to -1			144	152					100	98		
1 to 14	504	-[a]	-	411	388	374	340	87	250	262	291	254

[a] Single survivor died on Day 1 of the observation period

TABLE 7

Water consumption

Period of consumption (Day)	Water consumption (g/rat/day)					
	1M (Control)	2M (5.75)	3M (2.44)	4M (0.515)	5M (1.06)	6M (1.49)
2	-	-	-	17	4	7
3	37	-	-	24	29	26
4	36	-	-	31	34	38
5	36	-	-	32	36	36
6	35	-	-	33	36	35
7	36	-	-	35	35	35
8	36	-	-	34	34	31
9	36	-	-	34	35	33
10	37	-	-	32	32	33
11	36	-	-	33	31	30
12	34	-	-	33	34	33
13	38	-	-	36	33	39
14	36	-	-	35	34	32
Cumulative (g/rat) 2 to 14	433	-	-	409	407	408

Period of Consumption (Day)	Water consumption (g/rat/day)					
	1F (Control)	2F (5.75)	3F (2.44)	4F (0.515)	5F (1.06)	6F (1.49)
2	-	-	14	19	4	9
3	27	4	23	21	26	39
4	26	9	25	20	26	44
5	31	9	29	24	28	35
6	32	6	31	23	29	35
7	27	19	27	24	25	35
8	27	13	30	16	24	29
9	30	15	26	26	28	32
10	35	28	25	23	27	28
11	26	10	24	22	25	33
12	28	6	25	17	24	22
13	33	11	27	26	26	27
14	35	11	28	27	32	28
Cumulative (g/rat) 2 to 14	357	141	334	288	330	396

TABLE 8

Macroscopic pathology

Group	Rat	Observation
1M (Control)	61	Lungs: patches of congestion azygous, two foci left lung, multiple foci right anterior and posterior lobe.
	62	Lungs: large dark focus right posterior lobe.
	63 - 65	No abnormalities detected.
2M (5.75)	71	Brown staining around snout and jaws. Clear discharge from nose. Lungs: severe congestion all lobes. Kidneys: slightly large. Intestines: congested.
	72	Brown staining around snout and jaws. Clear discharge from nose. Lungs: severe congestion all lobes. Intestines: congested.
	73	Brown staining around snout and jaws. Clear discharge from nose. Lungs: severe congestion all lobes. Intestines: congested.
	74	Brown staining around snout and jaws. Lungs: severe congestion all lobes. Intestines: congested.
	75	Brown staining around snout and jaws. Clear discharge from nose. Lungs: severe congestion all lobes. Kidneys: slightly large. Intestines: congested.
3M (2.44)	81	Brown staining around snout and jaws and forepaws. Compound on fur. Lungs: severe congestion all lobes, clear frothy fluid from trachea. Brain: minimal congestion. Intestines: congested.
	82	Crusty brown staining around snout and jaws and forepaws. Red discharge from snout and jaws. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy liquid from trachea. Stomach: gas filled. Intestines: gas filled.
	83	Crusty brown staining around snout and jaws and forepaws. Yellow staining UG region. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy fluid from trachea. Intestines: congestion small intestines.
	84	Crusty brown staining around snout and jaws and forepaws. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy fluid from trachea.
	85	Crusty brown staining around snout and jaws and forepaws. Red discharge from snout and mouth. Slight feel of compound on fur. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy fluid from trachea. Stomach: gas filled.

UG Urogenital

TABLE 8

(Macroscopic pathology – continued)

Group	Rat	Observation
4M (0.515)	91 - 95	Lungs: pale raised areas all lobes.
5M (1.06)	21 - 24	No abnormalities detected.
	25	Lungs: pale raised areas all lobes.
6M (1.49)	1	No abnormalities detected.
	2	Lungs: pale cream coloured raised areas all lobes.
	3 – 4	No abnormalities detected.
	5	Lungs: pale raised areas all lobes.

TABLE 8

(Macroscopic pathology - continued)

Group	Rat	Observation
1F (Control)	66 - 70	No abnormalities detected.
2F (5.75)	76	Brown staining around snout and jaws. Clear discharge from snout and jaws. Lungs: severe congestion all lobes. Kidneys: slightly large. Intestines: congested.
	77	Brown staining around snout and jaws. Clear discharge from nose. Lungs: severe congestion all lobes. Kidneys: slightly large. Intestines: congested.
	78	Crusty brown staining around snout, jaws and forepaws. Yellow staining UG region. Lungs: severe congestion all lobes. Adrenals: right gland 2x longer than left. Intestines: gas filled areas of distension and congestion. Mesenteric lymph nodes: dark and enlarged.
	79	Yellow/brown staining UG region. Lungs: large areas of severe congestion all lobes. Raised hardened area (14mm x 9mm) left lung, raised hardened area pale (13mm x 18mm) right posterior.
	80	Crusty brown staining around snout, jaws and forepaws. Lungs: severe congestion all lobes. Stomach: gas filled. Intestines: gas filled.
3F (2.44)	86	Crusty brown staining around snout, jaws and forepaws. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy liquid from trachea. Stomach: gas filled. Intestines: gas filled.
	87	Crusty brown staining around snout, jaws and forepaws. Red discharge from snout and mouth. Compound on fur. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy fluid from trachea. Stomach: gas filled. Intestines: congested.
	88	Lungs: pale raised areas all lobes.
	89	Crusty brown staining around snout, jaws and forepaws. Brain: minimal congestion. Lungs: severe congestion all lobes, clear frothy liquid from trachea.
	90	Lungs: pale raised areas all lobes. Ovaries: 4 masses 5 × 5 mm (approx) in left horn of uterus, 1 mass on right side.

UG Urogenital

TABLE 8

(Macroscopic pathology – continued)

Group	Rat	Observation
4F	96	No abnormalities detected.
(0.515)	97 - 99	Lungs: pale raised areas all lobes.
	100	No abnormalities detected.
5F	26 - 30	No abnormalities detected.
(1.06)		
6F	6	Lungs: pale raised areas left lung and right anterior lobe.
(1.49)	7 – 8	No abnormalities detected.
	9	Kidneys: right kidney flattened with small area of hard yellow tissue.
	10	Lungs: pale raised areas all lobes.

TABLE 9

Organ weights

Group	Rat	Organ weight (g)					
		Lung		Liver		Kidneys	
		Decedent	Survivor	Decedent	Survivor	Decedent	Survivor
1M (Control)	61		1.65		17.63		2.71
	62		1.84		17.70		2.86
	63		1.84		15.94		2.59
	64		1.98		18.87		3.06
	65		1.61		19.07		2.89
	Mean	-	1.78	-	17.84	-	2.82
	sd	-	0.152	-	1.250	-	0.180
2M (5.75)	71	4.35		15.13		3.27	
	72	4.79		15.55		2.68	
	73	4.40		15.50		2.80	
	74	4.32		15.85		2.86	
	75	4.65		15.66		3.05	
	Mean	4.50	-	15.54	-	2.93	-
	sd	0.207	-	0.265	-	0.231	-
3M (2.44)	81	3.17		12.11		2.41	
	82	4.43		11.09		2.71	
	83	4.56		12.02		2.31	
	84	3.63		10.38		2.15	
	85	3.36		12.07		2.73	
	Mean	3.83	-	11.53	-	2.46	-
	sd	0.630	-	0.772	-	0.253	-
4M (0.515)	91		1.61		14.53		2.31
	92		1.72		15.16		2.31
	93		1.74		15.85		2.34
	94		1.74		14.48		2.44
	95		1.68		16.47		2.60
	Mean	-	1.70	-	15.30	-	2.40
	sd	-	0.055	-	0.860	-	0.124
5M (1.06)	21		1.68		13.30		2.21
	22		1.72		13.74		2.62
	23		1.90		15.54		2.26
	24		1.78		14.67		2.34
	25		1.67		15.27		2.34
	Mean	-	1.75	-	14.50	-	2.35
	sd	-	0.094	-	0.964	-	0.159
6M (1.49)	1		1.77		14.57		2.39
	2		1.87		15.45		2.71
	3		1.67		12.89		2.42
	4		1.82		17.78		2.40
	5		1.83		16.35		2.32
	Mean	-	1.79	-	15.41	-	2.45
	sd	-	0.077	-	1.841	-	0.151

TABLE 9

(Organ weights – continued)

Group	Rat	Organ weight (g)					
		Lung		Liver		Kidney	
		Decedent	Survivor	Decedent	Survivor	Decedent	Survivor
1F (Control)	66		1.39		11.71		1.71
	67		1.46		10.11		1.51
	68		1.46		10.80		2.00
	69		1.35		10.47		1.67
	70		1.29		11.40		1.80
	Mean	-	1.39	-	10.90	-	1.74
	sd	-	0.073	-	0.657	-	0.180
2F (5.75)	76	3.49		10.42		2.56	
	77	4.01		11.13		2.47	
	78	4.27		10.03		1.90	
	79		2.84		6.73		1.23
	80	3.53		8.41		1.50	
	Mean	3.83	2.84	10.00	6.73	2.11	1.23
	sd	0.379	-	1.152	-	0.499	-
3F (2.44)	86	3.97		9.13		2.16	
	87	4.11		9.36		2.06	
	88		1.73		10.40		1.55
	89	3.73		10.06		2.00	
	90		1.71		11.41		1.73
	Mean	3.94	1.72	9.52	10.91	2.07	1.64
	sd	0.192	-	0.484	-	0.081	-
4F (0.515)	96		1.18		6.99		1.57
	97		1.38		8.97		1.73
	98		1.36		9.91		1.65
	99		1.69		10.61		1.89
	100		1.33		8.63		1.61
	Mean	-	1.39	-	9.02	-	1.69
	sd	-	0.186	-	1.378	-	0.126
5F (1.06)	26		1.46		10.20		2.22
	27		1.47		9.75		1.88
	28		1.32		9.37		1.70
	29		1.59		8.96		1.70
	30		1.39		9.32		1.72
	Mean	-	1.45	-	9.52	-	1.84
	sd	-	0.101	-	0.472	-	0.223
6F (1.49)	6		1.45		8.51		1.62
	7		1.44		8.23		1.66
	8		1.60		9.46		1.62
	9		1.48		9.61		1.50
	10		1.76		8.75		1.75
	Mean	-	1.55	-	8.91	-	1.63
	sd	-	0.136	-	0.600	-	0.090

VII. APPENDIX 1

Certificates of Analysis for Diet

SDS

Special Quality Control
Certificate of Analysis

PRODUCT: RM1 (E) SQC
BATCH NO: PREMIX BATCH NO: 305
DATE OF MANUFACTURE: 08-JUL-98

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	10.6	%	Fluoride	10	mg/kg	1.0 mg/kg
Crude Fat	2.4	%	Nitrate as NaNO3	14	mg/kg	1.0 mg/kg
Crude Protein	14.8	%	Nitrite as NaNO2	2.9	mg/kg	1.0 mg/kg
Crude Fibre	4.1	%	Lead	0.45	mg/kg	0.25 mg/kg
Ash	4.6	%	Arsenic	Non Detected	mg/kg	0.2 mg/kg
Calcium	0.63	%	Cadmium	0.07	mg/kg	0.05 mg/kg
Phosphorus	0.51	%	Mercury	Non Detected	mg/kg	0.01 mg/kg
Sodium	0.21	%	Selenium	0.05	mg/kg	0.05 mg/kg
Chloride	0.30	%				
Potassium	0.70	%				
Magnesium	0.16	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	122	mg/kg				
Copper	10	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	50	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/k
Zinc	45	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/k
			Lindane	Non Detected	mcg/kg	10.0 mcg/k
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/k
			Malathion	20	mcg/kg	20.0 mcg/kg
Vitamin A	4.2	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	39	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	2.50	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	50	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed .....
Dated 31/7/98

VII. APPENDIX 1

(Certificates of Analysis for Diet - continued)

SDS

Special Quality Control
Certificate of Analysis

PRODUCT: RM1 (E) SQC
BATCH NO: PREMIX BATCH NO: 370
DATE OF MANUFACTURE: 28-AUG-98

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	9.3	%	Fluoride	5	mg/kg	1.0 mg/kg
Crude Fat	2.7	%	Nitrate as NaNO3	21	mg/kg	1.0 mg/kg
Crude Protein	14.6	%	Nitrite as NaNO2	3.1	mg/kg	1.0 mg/kg
Crude Fibre	4.3	%	Lead	0.31	mg/kg	0.25 mg/kg
Ash	5.0	%	Arsenic	Non Detected	mg/kg	0.2 mg/kg
Calcium	0.83	%	Cadmium	0.05	mg/kg	0.05 mg/kg
Phosphorus	0.48	%	Mercury	Non Detected	mg/kg	0.01 mg/kg
Sodium	0.20	%	Selenium	0.05	mg/kg	0.05 mg/kg
Chloride	0.48	%				
Potassium	0.68	%				
Magnesium	0.16	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	143	mg/kg				
Copper	11	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	52	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/k
Zinc	39	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/k
			Lindane	Non Detected	mcg/kg	10.0 mcg/k
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/k
			Malathion	Non Detected	mcg/kg	20.0 mcg/kg
Vitamin A	4.2	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	38	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	2.50	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	200	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed
Dated 28/9/98

VII. APPENDIX 1

(Certificates of Analysis for Diet - continued)

SDS

Special Quality Control
Certificate of Analysis

PRODUCT: RM1 (E) SQC
BATCH NO: PREMIX BATCH NO: 391
DATE OF MANUFACTURE: 16th September 1998

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	10.0	%	Fluoride	5	mg/kg	1.0 mg/kg
Crude Fat	2.6	%	Nitrate as NaNO3	17	mg/kg	1.0 mg/kg
Crude Protein	15.2	%	Nitrite as NaNO2	3.0	mg/kg	1.0 mg/kg
Crude Fibre	4.1	%	Lead	Non detected	mg/kg	0.25 mg/kg
Ash	4.9	%	Arsenic	Non detected	mg/kg	0.2 mg/kg
Calcium	0.71	%	Cadmium	Non detected	mg/kg	0.05 mg/kg
Phosphorus	0.49	%	Mercury	Non detected	mg/kg	0.01 mg/kg
Sodium	0.22	%	Selenium	Non detected	mg/kg	0.05 mg/kg
Chloride	0.49	%				
Potassium	0.70	%				
Magnesium	0.16	%	Total Aflatoxins	Non detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	131	mg/kg				
Copper	12	mg/kg	Total P.C.B	Non detected	mcg/kg	10.0 mcg/kg
Manganese	64	mg/kg	Total D.D.T	Non detected	mcg/kg	10.0 mcg/kg
Zinc	46	mg/kg	Dieldrin	Non detected	mcg/kg	10.0 mcg/kg
			Lindane	Non detected	mcg/kg	10.0 mcg/kg
			Heptachlor	Non detected	mcg/kg	10.0 mcg/kg
			Malathion	Non detected	mcg/kg	20.0 mcg/kg
Vitamin A	3.6	iu/g	Total Viable Organisms x 1000	Non detected	per grm	1000/g
Vitamin E	41	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	5.0	per grm	100/g
			Salmonellae Species	Non detected	per grm	Absent in 20 grm
			Entero Bacteriacese	Non detected	per grm	Absent in 20 grm
			Escherichia Coli	Non detected	per grm	Absent in 20 grm
			Fungal Units	Non detected	per grm	Absent in 20 grm
			Antibiotic Activity	Non detected		

Signed .....
Dated 8/10/98

VII. APPENDIX 1

(Certificates of Analysis for Diet - continued)

Special Quality Control Certificate of Analysis

PRODUCT: RM1 (E) SQC

BATCH NO: PREMIX BATCH NO: 506

DATE OF MANUFACTURE: 18-DEC-98

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	11.1	%	Fluoride	6	mg/kg	1.0 mg/kg
Crude Fat	4.0	%	Nitrate as NaNO3	17	mg/kg	1.0 mg/kg
Crude Protein	16.6	%	Nitrite as NaNO2	3.0	mg/kg	1.0 mg/kg
Crude Fibre	4.7	%	Lead	Non Detected	mg/kg	0.25 mg/kg
Ash	4.3	%	Arsenic	Non Detected	mg/kg	0.2 mg/kg
Calcium	0.88	%	Cadmium	0.10	mg/kg	0.05 mg/kg
Phosphorus	0.57	%	Mercury	0.03	mg/kg	0.01 mg/kg
Sodium	0.23	%	Selenium	0.12	mg/kg	0.05 mg/kg
Chloride	0.32	%				
Potassium	0.57	%				
Magnesium	0.15	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	126	mg/kg				
Copper	9	mg/kg	Total F.G.B	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	50	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/k
Zinc	45	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/k
			Lindane	Non Detected	mcg/kg	10.0 mcg/k
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/k
			Malathion	Non Detected	mcg/kg	20.0 mcg/kg
Vitamin A	4.7	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	45	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	Non Detected	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed .

Dated ....15/1/99.....

TROUW NUTRITION

VII. APPENDIX 1

(Certificates of Analysis for Diet - continued)

Special Diets Services

Special Quality Control Certificate of Analysis

PRODUCT: RMI (E) SQC

BATCH NO: PREMIX BATCH NO: 506

DATE OF MANUFACTURE: 29-JAN-99

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	11.1	%	Fluoride	7	mg/kg	1.0 mg/kg
Crude Fat	2.8	%	Nitrate as NaNO3	28	mg/kg	1.0 mg/kg
Crude Protein	15.7	%	Nitrite as NaNO2	2.7	mg/kg	1.0 mg/kg
Crude Fibre	5.2	%	Lead	Non Detected	mg/kg	0.25 mg/kg
Ash	4.7	%	Arsenic	Non Detected	mg/kg	0.2 mg/kg
Calcium	0.66	%	Cadmium	0.15	mg/kg	0.05 mg/kg
Phosphorus	0.49	%	Mercury	Non Detected	mg/kg	0.01 mg/kg
Sodium	0.26	%	Selenium	0.07	mg/kg	0.05 mg/kg
Chloride	0.40	%				
Potassium	0.69	%				
Magnesium	0.18	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1,G2
Iron	157	mg/kg				
Copper	11	mg/kg	Total P.C.B.	Non Detected	mcg/kg	10.0 mcg/kg
Manganese	60	mg/kg	Total D.D.T	Non Detected	mcg/kg	10.0 mcg/kg
Zinc	60	mg/kg	Dieldrin	Non Detected	mcg/kg	10.0 mcg/kg
			Lindane	Non Detected	mcg/kg	10.0 mcg/kg
			Heptachlor	Non Detected	mcg/kg	10.0 mcg/kg
			Malathion	Non Detected	mcg/kg	20.0 mcg/kg
Vitamin A	3.8	iu/g	Total Visble Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	42	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Entero Bacteriaceae	Non Detected	per grm	Absent in 20 grm
			Escherichia Coli	Non Detected	per grm	Absent in 20 grm
			Fungal Units	Non Detected	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed

Dated 17/2/99

TROUW NUTRITION

VII. APPENDIX 2

Certificate of Analysis for drinking water

ANALYTICAL DATA SUMMARY SHEETS

Huntingdon North Public Water Supply Zone

Population:- **01-Jan-99 - 31-Mar-99** Grid Ref:-

Parameter Ref	Name	PCV	Units	Number of samples		% samples contravening PCV	Concentration or Value (all samples) Minimum	Mean	Maximum
A001	Colour	20	Pt/Co	3	R	0	2.1	2.67	3.5
A002	Turbidity	4	FTU	12		0	0.15	< 0.271	0.88
A03a	Odour - Nature	-	-	12		-	1	1	1
A03b	Odour - Intensity	-	-	12		-	1	1	1
A04a	Taste - Nature	-	-	12		-	1	1	1
A04b	Taste - Intensity	-	-	12		-	1	1	1
A005	Temperature	25	°C	20		0	4.9	7.67	12.2
A006	Hydrogen ion (pH)	5.5 - 9.5	pH	12	R	0	7.81	7.87	7.96
A007	Sulphate	250	mg/l	1		0	134	134	134
A008	Magnesium	50	mg/l	1		0	8.05	8.05	8.05
A009	Sodium	150	mg/l	1		0	48.6	48.6	48.6
A09A	Sodium 80*	150	mg/l	3		0	0	65.5	0
A010	Potassium	12 (15)	mg/l	11	X	0	6.89	8.09	8.64
A011	Dry Residues	1500	mg/l	1		0	640	640	640
A012	Nitrate	50	mg/l	3		0	32.4	33.8	35.6
A013	Nitrite	0.1	mg/l	11	I	0	< 0.01	< 0.015	0.049
A014	Ammonium	0.5	mg/l	3		0	0.219	0.228	0.232
A016	Oxidisability	5	mg/l	1		0	1.72	1.72	1.72
A017	Total organic carbon	-	mg/l	1		-	3.99	3.99	3.99
A021	Aluminium	200	µg/l	4	R	0	< 10	< 10	< 10
A022	Iron	200	µg/l	3	R U	0	< 10	< 11.7	13
A023	Manganese	50	µg/l	4	R	0	< 2	< 2	< 2
A024	Copper	3000	µg/l	3	R	0	< 5	< 99.3	298
A025	Zinc	5000	µg/l	3	R	0	< 6	< 8.71	14.1
A026	Phosphorus	2200	µg/l	10		0	540	546	620
A027	Fluoride	1500	µg/l	1		0	250	250	250
A028	Silver	10	µg/l	1		0	< 1	< 1	< 1
B001	Arsenic	50	µg/l	1		0	1.49	1.49	1.49
B002	Cadmium	5	µg/l	1		0	< 0.4	< 0.4	< 0.4
B004	Chromium	50	µg/l	1		0	< 1.1	< 1.1	< 1.1
B005	Mercury	1	µg/l	1		0	< 0.1	< 0.1	< 0.1
B006	Nickel	50	µg/l	1		0	3.14	3.14	3.14
B007	Lead	50	µg/l	3	R	0	< 1.9	< 1.9	< 1.9
B008	Antimony	10	µg/l	1		0	0.49	* 0.49	0.49
B009	Selenium	10	µg/l	1		0	1.1	1.1	1.1
P014	Chlorotoluron	0.1	µg/l	4		0	< 0.02	< 0.02	< 0.02
P032	Diuron	0.1	µg/l	4		0	< 0.02	< 0.02	< 0.02
P048	Isoproturon	0.1	µg/l	4		0	< 0.02	< 0.022	0.03
P051	Linuron	0.1	µg/l	4		F 0	< 0.02	< 0.02	< 0.02
P113	Monuron	0.1	µg/l	4		0	< 0.02	< 0.02	< 0.02
P074	2,3,6 - TBA	0.1	µg/l	3		0	< 0.02	< 0.02	< 0.02
P020	2,4 - D	0.1	µg/l	3		0	< 0.05	< 0.05	< 0.05
P076	2,4,5 - T	0.1	µg/l	3		0	< 0.05	< 0.05	< 0.05
P006	Bentazone	0.1	µg/l	3		0	< 0.02	< 0.02	< 0.02
P025	Dicamba	0.1	µg/l	3		0	< 0.05	< 0.05	< 0.05
P026	Dichloroprop	0.1	µg/l	3		0	< 0.02	* < 0.02	< 0.02
P054	MCPA	0.1	µg/l	3		0	< 0.02	< 0.02	< 0.02
P055	MCPB	0.1	µg/l	3		0	< 0.05	< 0.05	< 0.05
P053	MCPP(Mecoprop)	0.1	µg/l	3		0	< 0.02	< 0.02	< 0.02
P004	Atrazine	0.1	µg/l	5		0	< 0.02	< 0.02	< 0.02
P070	Prometryne	0.1	µg/l	5		0	< 0.02	< 0.02	< 0.02
P066	Propazine	0.1	µg/l	5		0	< 0.02	< 0.02	< 0.02
P073	Simazine	0.1	µg/l	5		0	< 0.02	< 0.02	< 0.02

Page 1

VII. APPENDIX 2

(Certificate of Analysis for drinking water – continued)

ANALYTICAL DATA SUMMARY SHEETS

Huntingdon North Public Water Supply Zone

Population:- 01-Jan-99 - 31-Mar-99 Grid Ref:-

Parameter Ref	Name	PCV	Units	Number of samples	% samples contravening PCV	Concentration or Value (all samples) Minimum	Mean	Maximum
P077	Terbutryne	0.1	µg/l	5	0	< 0.02	< 0.02	< 0.02
P132	Trietazine	0.1	µg/l	5	0	< 0.02	< 0.02	< 0.02
B610	Pesticides - Total	0.5	µg/l	5	0	0	0.01	0.02
C001	Total Coliforms	0	No/dl	22	0	0	0	0
C002	Faecal Coliforms	0	No/dl	22	0	0	0	0
C008	Colony Count 1Day @ 37°C	-	No/ml	22	-	0	1.73	5
C012	Colony Count 7Day @ 22°C	-	No/ml	22	-	1	44.2	208
C910	Chlorine Total	-	mg/l	22	-	0.15	0.599	0.9
D01a	Conductivity - M12	1500	µs/cm	12	0	821	827	832
D02a	Chloride - M12	400	mg/l	1	0	65.9	65.9	65.9
D03a	Calcium - M12	250	mg/l	1	0	140	140	140
D05a	Boron - M12	2000	µg/l	1	0	244	244	244
D06a	Barium - M12	1000	µg/l	1	0	14.8	14.8	14.8
D08a	Tetrachloromethane - M12	3	µg/l	3	0	0.1	0.1	0.1
D09a	Trichloroethene - M12	30	µg/l	3	0	0.4	0.4	0.4
D10a	Tetrachloroethene - M12	10	µg/l	3	0	0.3	0.3	0.3
E001	Hardness as Ca - Min	-	mg/l	1	-	149	149	149
E002	Alkalinity - HCO3 - Min	-	mg/l	1	-	272	272	272

Notes

PCV - Prescribed concentration or value
M12 - Rolling 12 month mean
M3 - Rolling 3 month mean
Min - PCV is a minimum only where the water is softened
U - Undertaking
X - Relaxation (relaxed value in brackets under PCV column)
R - Reduced sampling frequency
I - Increased sampling frequency
PAH - Polycyclic aromatic hydrocarbons

Sodium 80* - the 80th percentile of the last 3 years of sodium results

Page 2

VII. APPENDIX 2

(Certificate of Analysis for drinking water – continued)

Anglian Water Services Ltd.
Drinking Water Register
HUNTINGDON NORTH, Water Supply Zone

Field(s): FENLAND **Date:** 1 January 1999

Map Ref:

Grid Reference:

Population Served:

Previous Name: HUNTINGDON NORTH

County Council: CAMBRIDGESHIRE

District Council: HUNTINGDONSHIRE DISTRICT COUNCIL

District Health Authority: CAMBRIDGE AND HUNTINGDON (WILL UNDERGO A NAME CHANGE IN 1999)

Water Treatment Works: GRAFHAM

VII. APPENDIX 2

(Certificate of Analysis for drinking water – continued)

Anglian Water Services Ltd.
Drinking Water Register

HUNTINGDON NORTH Water Supply Zone

Number of Storage Points Serving The Zone: SEVEN
Number of Booster Pumping Stations: ONE

Storage Points	WTW / Final Water PL Supplying Res / TWR and % Composition		Storage Capacity (tons)
BUCKDEN RESERVOIR	GRAFHAM	100.0	8.570
GODMANCHESTER RESERVOIR	GRAFHAM	100.0	2.600
GRAFHAM TOWER	GRAFHAM	100.0	0.070
PERRY TOWER	GRAFHAM	100.0	0.340
SAPLEY RESERVOIR 1	GRAFHAM	100.0	9.000
SAPLEY TOWER	GRAFHAM	100.0	1.130
THREE SHIRES TOWER	GRAFHAM	100.0	0.340

Comments:
GRAFHAM TOWER & PERRY TOWER ARE SUPPLIED BY BUCKDEN RES. SAPLEY RES No.1 AND SAPLEY TOWER ARE SUPPLIED FROM SAPLEY RES No.2.

RELAXATIONS

Reference No.	Parameters	Works	Expiry/Review Date
144 ANG	Potassium P	GRAFHAM	31 December 1999

SECTION 19(1) (B) UNDERTAKINGS

Parameter	Water Treatment Works	Supply Zone Completion Date
Distribution		
IRON	REHABILITATE UNLINED IRON MAINS	31 March 2000

Page 2

VII. APPENDIX 3

(Certificate of analysis Microbiological analysis of animal drinking water)

Source of water sample (s):	Huntingdon Research Centre, Building Y13,	
Date sampled and tested :	(1) September 1998 Rm 008 (2) September1998 Rm 006 (3) 20 January 1999 Rm 007 (4) 20 January 1999 Rm 005 (5) 11 May 1999 Rm 008 (6) 11 May 1999 Rm 006	
Test procedure :		
Research Laboratory	Huntingdon Research Centre Department of Cellular Sciences Woolley Road Huntingdon Cambridgeshire PE17 5HS ENGLAND	
RESULTS	**Count**	**Specification**
Total viable count for aerobic bacteria :	(1) 0 cfu/ml (22°C) (2) 0 cfu/ml (22°C) (3) 2 cfu/ml (22°C) (4) 1 cfu/ml (22°C) (5) 0 cfu/ml (22°C) (6) 0 cfu/ml (22°C)	$<10^4$ cfu/ml (22°C)
	(1) 0 cfu/ml (37°C) (2) 0 cfu/ml (37°C) (3) 3 cfu/ml (37°C) (4) 5 cfu/ml (37°C) (5) 0 cfu/ml (37°C) (6) 0 cfu/ml (37°C)	$<10^2$ cfu/ml (37°C)
Total viable count for coliform bacteria :	Samples1-6 <1 cfu/100ml	<1 cfu/100ml
Total viable count for *E.coli* :	Samples1-6 <1 cfu/100ml	<1 cfu/100ml
CONCLUSION	All samples showed satisfactory microbiological quality.	
Results reviewed by : **Head, Microbiology**	**Signature :** **Date :** 07/01/2000	

cfu - colony forming unit